UNCLASSIFIED

AD NUMBER

AD833600

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; NOV 1966. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Releases Branch, Frederick, MD 21701.

AUTHORITY

Fort Detrick/SMUFD ltr dtd 15 Feb 1972

THIS PAGE IS UNCLASSIFIED

AD833600

TRANSLATION NO. 1916

XXXXXXX- CA-18-064-D6-00030(A)

DATE: 2 November 1966

DDC AVAILABILITY NOTICE

-1/3

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

1911

CA-18-064-D6-00030(A)

T-449-1

2 November 1966

IMMUNOLOGICAL RESEARCH ON BASIC SPECIFIC AND NON-SPECIFIC PROTEIN ANTIGENS OF THE TYPE STREPTOCOCCUS PYOGENES (GROUP A)

IMMUNOLOGICAL RESEARCH ON BASIC SPECIFIC AND NON-SPECIFIC PROTEIN ANTIGENS OF THE TYPE STREPTOCOCCUS PYOGENES (GROUP A)

Annales De L'Institut Pasteur
(Annals of the Pasteur Institute)
No. 109, pages 479-486, 1965

Wahl, R.,
Goichot, J. &
Drach, G.

The antigens previously studied by hemagglutination [16] and by diffusion [17] are studied here by immunoelectrophoresis.

APPARATUS AND METHODS.

1. Extracts - The extracts used were described in previous publications [15, 17]. They were obtained commencing from a group A strain of streptococcus, of type 24 (A 18 t 24). It involves:

a) Acid-alcohol extract (extract Γ) [1] and its chromatographic fractions [16, 17].

b) Acidic extract prepared by the action of M/S hydrochloric acid at 90° upon bacteria, following Lancefield's method [9].

These extracts contain nucleic acids and polysaccharides.

Protein analysis was done by the Folin-Lowry method, that of the glucides by the reaction of anthrone, that of the nucleic acids by the spectrophotometer.

The extracts were concentrated by polyethylene glycol, either to 2 mg, or to 6 mg of protein per millilitre.

They were dialysed each time against the buffer which in the corresponding experiment was required for electrophoresis.

2. Serums - Serums of type 24 obtained by intravenous immunization of rabbits with streptococci of the strain A 18 t 24, killed by heat. The serum obtained was termed "non-absorbed serum 24."

The same serum 24 absorbed by a heterologic strain.

Serum 154 obtained by injection of extrat Γ with Freund's adjuvant.

3. Immunoelectrophoresis -- They were carried out in gelose on slides following the microtechnique described by Grabar and Williams [5], Grabar et al. [4] as well as by Scheidegger [13], using modifications which are described below, in the case where the buffer used was at a pH less than 6.7 or greater than 8.2.

We used an agar gel, neutral gelose, at a 0.75% concentration to reduce convection currents, and with an addition of 1/1000 of methiolate.

Voltage was 120 V for a length of 7.5 cm. i.e. 16 V/cm, 50 mA, electrophoresis times being in general two and a half hours.

Seven different pH's were used which were obtained with the following buffers:

a) pH 5.3 and 6.1 with an acetate buffer

b) pH 6.7 and 8.2 with a veronal buffer

c) pH 9.5 , 10.5, 10.9 with a sodium glycine buffer.

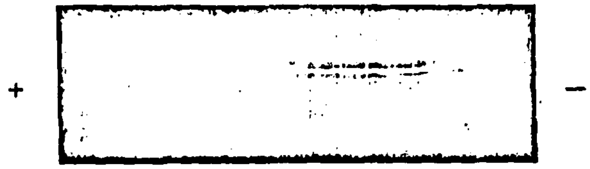

Fig. 1.

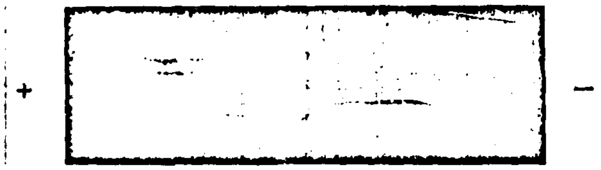

Fig. 2.

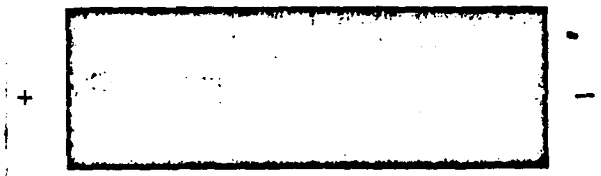

Fig. 3.

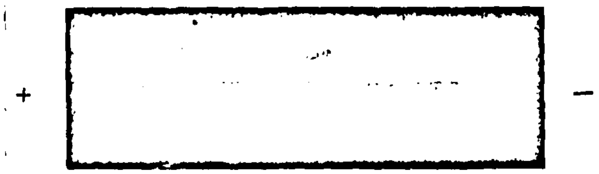

Fig. 4

Plate 1

Figure 1 - Immunoelectrophoresis at pH 5.3 in the presence of non absorbed type 24 serum.

<u>Top reservoir</u>: non purified acid 24 extract (Lancefield), concentration 6 mg/ml. The dense arc of the type specific protein as well as the two arcs joined at one of their extremities, corresponding to one or two non type specific antigens, are situated on the side of the negative pole.

Figure 2 - Immunoelectrophoresis at pH 9.5 in the presence of type 24 non-absorbed serum.

<u>Top reservoir</u>: non-purified acid 24 extract

(Lancefield), concentration 6 mg/ml.

Bottom reservoir Γ_{24} extract, concentration 2 mg/ml. For the two extracts: the dense band of the specific protein of the type is situated towards the positive pole. The thin streak, situated close to the spout for the serum, is drawan out toward the two poles. The thin band due to a non type specific antigen is situated toward the negative pole.

Figure 3 - Immunoelectrophoresis at pH 10.5 with an ionic force of 0.2 in the presence of type 24 non absorbed serum.

Top reservoir: non-purified acid 24 extract (Lancefield), concentration 6 mg/ml.

Bottom reservoir: Γ_{24} extract, concentration 6 mg/ml. For this pH and ionic force the typical non-specific antigen in the extracts is in the neighborhood of its isoelectric point.

Figure 4 - Immunoelectrophoresis of the III α fraction at pH 8.4 in presence of serum 1954.

The streaks are blurred and very drawn out. Little can be distinguished clearly.

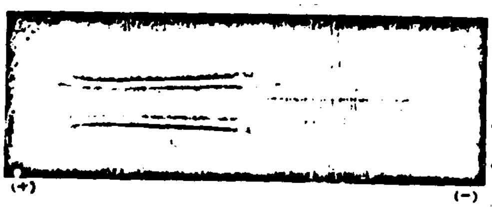

FIG. 5.

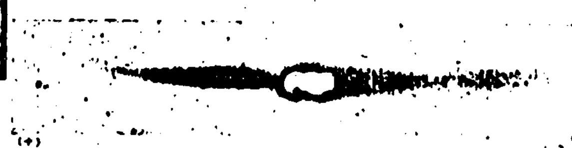

FIG. 7.

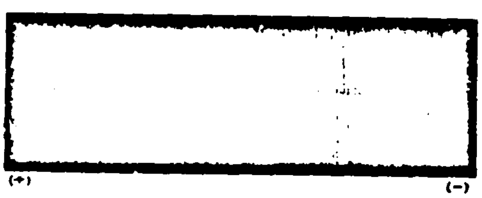

FIG. 6.

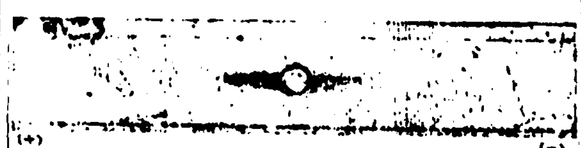

FIG. 8.

Plate 2

Figure 5 - Immunoelectrophoresis at pH 7.4 in presence of non-absorbed serum 24.

Top reservoir) Acid 24 extract non-purified (Lancefield)
Bottom reser-) Concentration 6 mg/ml.
voir)

The thick curve of the type specific protein is situated close to the position pole. The fine streaks corresponding to the polysaccharide and the non-specific antigen are situated toward the negative pole.

Figure 6 - Immunoelectrophoresis at pH 6.1.

Top reservoir: non-purified acid 24 extract (Lancefield), concentration 6. mg/ml.

Bottom reservoir: non-purified acid 24 extract (Lancefield), concentration 2 mg/ml.

For this pH the smear of the protein M of the acid extract is not displaced. It is found near the isoelectric point. The smears corresponding to other antigens are only visible at a concentration of 6 mg/ml (and not at 2 mg/ml).

Figure 7. Electrophoresis at pH 6.1 of non-purified extract of acid 24. (Lancefield), concentration 2 mg/ml.

The position of the band shows that the M protein is found close to its isoelectric point. Bands corresponding to the non-type specific antigens are not found, because of too low a concentration of the extract.

The ionic strength of the buffers was 0.05 in all cases except in one experiment where a glycine buffer at pH 10.5 with an ionic strength of 0.2 was used, in an attempt to lower the isoelectric point of the proteins studied (Crowle [3]), which effectively gave the rejected result. The gelose was dissolved in each case by means of the buffer used for the electrophoresis, but diluted by half (0.025 M and 0.1 M).

When the pH of the buffer was below 6.5 in order to avoid liquifaction of the gelose by heating in an acidic medium, we used the method described by Grabar and Burtin [6] the agar being dissolved in redistilled water at the usual concentration of 0.75%. The pH of the gelose was then adjusted to the pH of the buffer used for electrophoresis by dipping the plate of gelose in redistilled water in the acetate buffer for 30 minutes.Electrophoresis was then performed in an acid medium, the slides finally being submerged for about 30 minutes in a bath of veronal buffer at pH 8.2 before depositing the serum.

For pH's about 8.6, the gelose was prepared directly in the buffer, but after electrophoresis the sheets were dipped in a bath of veronal buffer at pH 8.2, as above.

4. Electrophoresis: - The Γ extract and the non-purified acid extract (Lancefield) underwent electrophoresis for 2 ½ hours on agarose gel, using either an acetate buffer at pH's of 5.3 and 6.1 respectively, or a sodium glycine buffer at pH 9.5.

After electrophoresis, the antigens were fixed in the gel by a fixing mixture of 50% alcohol and /? - illegible, translator/ % acetic acid for 3 hours. The gelose was dried in an incubator and colored with either amidoschwarz /meaning not known - translator/ or with azocarmine /14/.

RESULTS

I - Γ_{24} Extract

1. Immunoelectrophoresis with non-absorbed serum 24.

Preliminary trials with preparations containing 1 to 2 mg/ml of proteins carried out at pH 8.2 and 8.6 gave:

a) an arc situated near the negative pole.

b) A thin blurred line, situated along the entire length of the serum groove and very close to it, being drawn equally towards the two poles.

Other smears seemed to be present but difficult to define. Stronger concentrations of antigens were, therefore, necessary.

Highly concentrated Γ_{24} extract (6 mg/ml of protein) was then submitted to electrophoresis at 7 different pH values (see APPARATUS AND METHODS) from 3.5 to 10.5 then placed with non-absorbed serum 24. This gave:

a) the dense arc already obtained with less concentrated Γ extract; its position varied with the pH. The antigen migrated toward the negative pole for pH values of 3.5 to 8.2 (plate 1, fig. 1) and toward the positive pole for pH 9.5 and at pH 10.5 (plate 1, fig. 2). This arc corresponds to a type specific antigen, as we shall see.

b) The thin and hazy line, which was also obtained with the less concentrated extract at pH 8.2 and 8.5 was not observed at pH 3.5 (plate 1, fig. 1) but only at pH 6.5 and above, including 10.5 (plate 1, fig. 2) (this shows the characteristics described above). Its appearance was the same at all pH. It corresponds to the group polysaccharide, because the polysaccharide purified by the Krause and MacCarthy method /8/ gives a similar line under the same experimental conditions.

c) A line situated for the most part towards the side of the negative pole and ending on this side with an arc observed at all the pH values used (from pH 3.5 to pH 10.5) for an ionic strength of 0.05 (plate 1, fig. 2).

But, in carrying out a new electrophoresis at pH 10.5 using a higher ionic strength in an attempt to lower the isoelectric point, no migration of the corresponding antigen occurred (plate 1, fig. 3) as the position of the smear showed. The smear, as we shall see, corresponds to one or two non type specific antigens, different from the group polysaccharides.

2. IMMUNOELECTROPHORESIS WITH ABSORBED SERUM 24

Only the dense arc is visible. This arc thus corresponds to the antigen specific to the type.

3. ELECTROPHORESIS - At pH 9.5 the Γ_{24} extract, at a protein concentration of 6 mg/ml, gives a dense band situated toward the positive pole which corresponds to the type-specific antigen, and two bands near the negative pole of which one corresponds to polysaccharide and the other to one or two non-type specific protein antigens (plate 2, fig. 7).

II - Fraction III α (plate 1, fig. 4).

Immunoelectrophoresis of the III α fraction with non-absorbed serum 24 gave no trace. With serum 154, one, two or three very fine smears, very drawn out equally toward the two poles.

One notes that the fraction III α antigens are very unstable, which may explain certain experimental difficulties.

III - ACID EXTRACT 24 NON-PURIFIED (LANCEFIELD)

1. IMMUNOELECTROPHORESIS WITH NON-ABSORBED SERUM 24

Like Γ extract with non-absorbed serum 24 it gives the thick arc of the M protein, the fine band of polysaccharide and the line attributed to one or two non-specific antigens.

The fact that the acid extract, according to Lancefield, contains antigens other than the M protein was already known [10, 15, 2].

These antigens were identified, as in the case of the Γ extract.

However, the M protein of the purified [10] or non-purified [9] acid extract behaves differently in immunoelectrophoresis than the M protein of the Γ extract.

There is an essential difference: the M protein of the acid extract migrates to the negative pole only up to pH 6.1 (plate 1, fig. 1). Commencing at pH 6.4 and up to pH 10.5 it migrates toward the positive pole (plate 2, figs. 5, 6). Now, we have seen that protein M of the Γ extract migrates to the negative pole up to pH 9 and toward the positive pole starting at pH 9.5

Heating in an acidic medium as used in the Lancefield method does not appear to be the only cause of this difference. In effect, Γ extract, treated as arc bacteria during extraction by the method of Lancefield (heating at 95° for 10 minutes at pH 2) again gives in immunoelectrophoresis at pH 8.2 a smear of type specific protein situated near the negative pole.

In contrast the polysaccharide and the one or more type specific antigens migrate in the same manner as those of Γ extract i.e. toward the negative pole up to pH 10.5. They no longer migrate at pH 10.5 when the ionic strength (0.2) is increased.

2. Electrophoresis - At pH 6.1 we verified that the Lancefield M protein does not migrate and in the experimental conditions is found at its isoelectric point (plate 2, fig. 8).

DISCUSSION

1. The Γ extract contained polynucleotides.

The fractions did not contain them. But the polynucleotides did not appear, under the conditions used, to give the precipitation trace nor to modify the migration of the proteins.

2. The identification of the streak corresponding to the type specific protein, in the electrophoresis of the Γ extract, was carried out as stated above.

This M protein of type 24 gave a single streak in immunoelectrophoresis, whereas in diffusion in gelose [1] at times it gave two streaks. As we saw previously, the significance of these two streaks is still uncertain.

The method of immunoelectrophoresis in gelose does not permit precise determination of the isoelectric point of a protein, but reversal of the direction of the M protein of Γ extract at pH 9.5 permits its location close to pH 9.

Now, the isoelectric point of the M protein of the Lancefield extract is definitely located at a very much lower pH; by the same method we were able to determine it as being about pH 6.1.

The M protein of the Γ extract is probably nearer to the pure state than that of the Lancefield extract, because the Γ extract is prepared by an easier method.

The M protein in its pure state is thus clearly basic, as has been indicated [1].

The M protein prepared by Kantor's method is also more acidic than the M protein of the Γ extract.

We note that our evaluation of the isoelectric point of the M protein of the Lancefield extract at 6.1 differs slightly from that of Lancefield and Perlmann [11] and from that of Zittle and Seibert [19] who determined it, using a Tiselius apparatus, as being at pH 5.3 for one type and 5.4 for the other. Two reasons may explain the difference with our results:

a) These authors used a buffer of ionic strength twice (0.1) ours, which could give a lower value for the isoelectric point of the protein.

b) They operated in a liquid medium, whereas in electrophoresis on gelose, the electroendosmosis could modify the migration of the protein.

3. The trace corresponding to the group polysaccharide

was also identified. During diffusion in gelose [17] precipitation of polysaccharide was not always observed, because it moved very rapidly until disappearing in the serum well and was produced only for certain concentrations of polysaccharide.

However, the trace was almost always observed during immunoelectrophoresis.

4. The non type specific protein antigens are different from the antigens described by Wilson and Wiley, antigen E, E4, glycerophosphate [18] which as we have seen [17] is not found in our preparations.

Further, we were not able to establish a parallel between the antigens discovered by us and those described by Pierce [12]. On the one hand his extracts, obtained by the Lancefield method, underwent fractioning with ammonium sulfate. Also his method of electrophoresis differed from ours.

The basic non type specific protein antigens which were identified previously by passive hemaglutination and diffusion in gelose were also shown by use of immunoelectrophoresis. This shows that they are even more basic than the M protein contained in the same extract.

However, the streaks corresponding to these antigens were not obtained in diffusion with serum 24, but only with serum 154, richer in corresponding antibodies. Perhaps the reason they were obtained by immunoelectrophoresis is that the antigen concentrations used were higher (6 mg/ml of protein) than those used in diffusion (1 to 3 mg/ml).

It is also possible that during diffusion certain antigens, having different electrophoretic mobilities, diffused with the same velocity.

Among the non type specific protein antigens, the IIIα fraction gave no streak with the absorbed serum 24, both in diffusion and in immunoelectrophoresis. On the contrary serum 154 gave a streak in diffusion and 1 to 3, dependent upon the case, very thin streaks during immunoelectrophoresis.

In previous publications [16, 17], we reported that this IIIα fraction, if it does not produce precipitant antibodies, provokes a retarded cutaneous allergy. This fraction was held responsible for the retarded allergy.

RESUME

Immunoelectrophoresis and electrophoresis have confirmed that acid-alcohol Γ extracts contained several type specific and non-specific protein antigens which were basic proteins. Their isoelectric point is above 8.5, while that of the non-type specific proteins was higher than that of the M protein.

They showed that the isoelectric point of M protein (about pH 9) extracted by the acid-alcohol method is very much greater than that of M protein extracted by the Lancefield method. This difference appears to indicate that the M protein is found in different states in the two extracts.

In contrast, the migration of the non-type specific antigens is the same in the Γ and Lancefield extracts.

Immunoelectrophoresis showed that the IIIα fraction, which is responsible for the retarded allergy, contains several antigens or several antigenic determinants.

SUMMARY

ELECTROPHORESIS AND IMMUNOELECTROPHORESIS OF ANTIGENIC COMPLEXES FROM GROUP A STREPTOCOCCI

It has been confirmed that acid-alcohol extracts contained several type specific and non specific proteic antigens, which were basic proteins.

ANNALES DE L'INSTITUT PASTEUR

The isoelectric point of these antigens was situated beyond pH 8.5. It w s higher for the non type specific proteins than for the M protein.

It has been shown that the value of the isoelectric point of the M protein was much greater when extracted by the acid-alcohol method than when extracted by the Lancefield method.

This seems to mean that the M protein is in a different state in one and the other extract.

On the contrary, the migration of the non type specific antigens was similar in the Γ extract and in the Lancefield extract.

It was shown by immunoelectrophoresis that fraction IIIα responsible for the delayed hypersensitivity contained either several antigens or several antigenic determinants.

BIBLIOGRAPHY

[1] CAYEUX (P.). Ann. Inst. Pasteur, 1963, 105, 1063.

[2] CAYEUX (P.). Ann. Inst. Pasteur, 1962, 103, 24.

[3] CROWLE (A.J.). Immunodiffusion, Academic Press. New York, 1961.

[4] GRABAR (P.), URIEL (J.) et COURCON (J.). Ann. Inst. Pasteur, 1960, 99, 13.

[5] GRABAR (P.) and WILLIAMS (C.A.Jr). Bioch. Biophys. Acta, 1955. 17, 67.

[6] GRABAR (P.) and BURTIN (P.). Immunoelectrophoretic analysis. Elsevier Publishing Cy, Amsterdam, Londres, New York, 1964.

[7] KANTOR (F.S.) and COLE (R.M.). J. exp. Med. 1960, 112, 77.

[8] KRAUSE (R.M.) Bact. Rev., 1963, 27, 369.

[9] LANCEFIELD (R. C.). J. exp. Med., 1928, 47, 91.

[10] LANCEFIELD (R. C.). J. exp. Med., 1928, 47, 469.

[11] LANCEFIELD (R. C.) and PERLMANN (G. E.). J. exp. Med., 1952, 96, 71.

[12] PIERCE, W.A. Jr). J. Bact., 1964, 88, 912.

[13] SCHEIDEGGER (J.J.). Int. Arch. Allergy, 1955, 7, 103.

[14] URIEL (J.) et GRABAR (P.) Ann. Inst. Pasteur, 1956, 90, 429.

[15] WAHL (R.), CAYEUX (P.) and DERLOT (E.). Ann. Inst. Pasteur, 1960, 99, 654.

[16] WAHL (R.) and DRACH (G.). Ann. Inst. Pasteur, 1964, 107, 809.

[17] WAHL (R.) and DRACH (G.). Ann. Inst. Pasteur, 1965, 108, 734.

[18] WILSON (A.T.) and WILEY (G. G.). J. exp. Med. 1963, 118, 521.

[19] ZITTLE (C.A.) and SEIBERT (F. B.). J. Immunol., 1942, 43, 47.